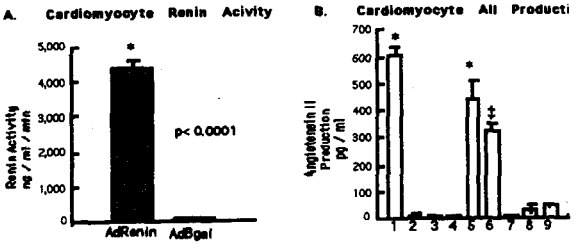
able to generate AII. (A): CM cultures were prepared from three day old Sprague Dawley neonates; infected with a total of 4×10^7 PFU/ml of either AdRe (n = 6) or Adβgal (n = 4) and culture media was collected after 72 H. Samples that received AdRe had significantly higher levels of measured Re activity as compared to controls ($p < 0.0001$). (B): Cultured CMs were infected with a total of 4×10^7 PFU/ml of Ad Re, alone or in combination with other Ad vectors carrying transgenes of RAS and/or ET systems: 1 = AdRe; 2 = AdAo; 3 = $AdAT_1$; 4 = AdET; 5 = AdRe + AdAo; 6 = AdRe + AdAo + $AdAT_1$; 7 = Adβgal + Tx; 8 = $AdAT_1$ + Tx; 9 = Adβgal. Tx = CMs treated with 10^{-6}M AII 24 H prior harvest.



Results revealed that all preparations infected with AdRe activated an endogenous RAS cascade as evidenced by substantial amounts of measured AII levels in the culture media (*$p < 0.0001$; ‡$p < 0.001$). We conclude that, when provided with an Re source, a local RAS is activated in CMs resulting in AII synthesis and release.

10:45

703-2 Proinflammatory Cytokines Antithetically Alter Expression of Disintegrin-Metalloprotease and Tissue Inhibitor of Metalloproteinase-3 in Neonatal Rat Cardiomyocytes

Y.Y. Li, C.F. McTiernan, A.M. Feldman. *University of Pittsburgh Medical Center, Pittsburgh, PA, USA*

Proinflammatory cytokines induce multiple gene responses in the myocardium. In order to identify these genes, we used mRNA differential display to study mRNAs that were isolated from cultured neonatal Sprague-Dawley rat cardiomyocytes treated with 100 units/ml tumor necrosis factor-alpha (TNFα) and 5 ng/ml interleukin 1-beta (IL-1β). Two functionally related genes were identified to be differentially regulated: disintegrin-metalloprotease (ADAM10) and tissue inhibitor of metalloproteinase-3 (TIMP3). Combined treatment of cultured neonatal rat cardiomyocytes with TNFα and IL-1β for 36 hours stimulated the expression of ADAM10, and at the same time suppressed the expression of TIMP3 as determined and quantified by reverse Northern blot, RNase protection assay and Northern blot analysis. Time course study with RNase protection assay and Northern blot analysis showed that the time-dependent induction of ADAM10 expression was caused largely by TNFα, but the decrease in TIMP3 expression was caused by IL-1β. As the production of both TNFα and IL-1β is elevated in patients with heart failure, the joint effect of these proinflammatory cytokines regulates the expression of ADAM10 and TIMP3 genes concomitantly. The resulting effect could lead to altered balance between protease and the protease inhibitor, and may play a role in the remodeling of the myocardium involving proinflammatory cytokines.

11:00

703-3 Cardiovascular Actions of Steroid Hormones: Purification and Sequencing of the First Putative Steroid Membrane Receptor

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For the past decade, nonclassical, rapid actions of steroid hormones including those on cardiovascular functions (e.g. immediate aldosterone action on baroreceptor activity and peripheral resistance, estrogen effects on coronary vascular tone and on calcium fluxes in vascular smooth muscle cells) have gained increasing scientific interest. These effects of steroids appear to be mediated by high affinity membrane steroid binding sites rather than by classical intracellular steroid receptors, with intracellular signalling involving calcium, phopholipase C, proteinkinase C and phosphoinositides. In search for the cloning of the first member from this new family of putative steroid receptors high affinity aldosterone and progesterone binding sites have been characterized in porcine liver by ^{3}H-steroid binding. Progesterone sites were purified by anion-exchange chromatography and functionally solubilized by cDNA sequence of a 28-kDa protein was determined in a porcine vascular smooth muscle cell library. The cDNA encodes for a protein of 194 amino acids with one transmembrane segment. A second progesterone-binding protein of 58 kDa was identified with an identical N-terminus. As tested by polyclonal antibodies, abundance of these sites is high in liver, kidney, lung and heart. This first putative steroid membrane receptor will serve as a template for the molecular analysis of membrane receptors specific for other steroids, and as a tool in the search for physiological and pathophysiological clues of rapid cardiovascular steroid action with potential therapeutic implications, e.g. vascular relaxation.

11:15

703-4 The Apoptosis Receptor Fas is Upregulated in Atrial Myocardium in Patients With Congestive Heart Failure

H. Schumann, D. Darmer, R. Pregla, K. Besler [1], H.R. Zerkowski [1], J. Holtz. *Institute of Pathophysiology, Martin-Luther-University, Halle, Germany,* [1] *Clinic of Cardiothoracic Surgery, Martin-Luther-University, Halle, Germany*

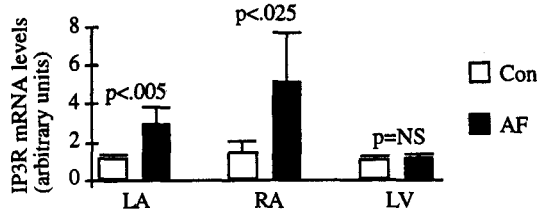
The endogenous program of cell death (apoptosis) of cardiocytes is elicited by oxidative stress and/or via activation of the cardiocyte Fas receptor by the Fas-ligand of activated T-cells or neutrophils. Severe overstretching of papillary muscles in vitro enhances myocardial Fas receptor expression, suggesting that cardiac overload may sensitize the myocardium for apoptotic cardiocyte losses due to the oxidative stress of ischemia/reperfusion. We wondered whether cardiac overload in vivo can enhance Fas expression in human myocardium. Therefore, right atrial appendages were obtained by informed consent from 47 consecutive cardiac surgery patients of either NYHA class 1 (n = 9) or NYHA class 3.5–4 (n = 38) during the installation of cardiopulmonary bypass. In the extracted atrial ribonucleic acid (RNA), Fas receptor messenger RNA (mRNA) was quantified by a standard calibrated, competitive reverse transcription polymerase chain reaction, using GGACCCAGAATACCAAGTG (sense) and CTGTTCTGCTGTGTCTTGG (antisense) primers. In atria from patients with NYHA class 3.5–4, Fas mRNA was 3.02 ± 0.26 (SEM) amol/μg RNA, significantly higher than in NYHA class 1 (1.50 ± 0.39; $p < 0.01$). Within NYHA class 3.5–4, patients under ACE inhibitor therapy had similar Fas mRNA levels (2.99 ± 0.39; n = 17) as those patients without this therapy (3.04 ± 0.35; n = 21). We propose that the enhanced Fas receptor expression in severe heart failure renders the failing human myocardium more susceptible to apoptotic cardiocyte losses during hypoperfusion, and that these losses contribute to an accelerated transition into terminal failure.

11:30

703-5 Selective Up Regulation of Inositol 1,4,5-Trisphosphate Receptor/Calcium Release Channel in Atrial Fibrillation

L.O. Go, C.C. Ward, P. Rosenberg, A. Schussheim, A.J. Gomes, A.R. Marks. *Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY, USA*

Intracellular Ca^{2+} release is mediated by the ryanodine receptor (RyR) and inositol 1,4,5-trisphosphate receptor (IP3R) channels, and their ventricular expression is differentially regulated during heart failure (*JCI* 95: 888, 1995). Since elevated intracellular Ca^{2+} is implicated in genesis of cardiac arrhythmias, we examined whether such regulation also occurs during atrial fibrillation (AF). Total RNA was isolated from the left and right atria (LA & RA) and left ventricle (LV) obtained from 45 cardiac transplant patients – 7 had history of AF, while 38 who did not served as controls. mRNA levels of RyR and IP3R were quantified via Northern and slot blot hybridizations with specific cDNA probes and normalized to 28S ribosomal RNA levels. Northern analyses detected a single ~ 16 kb RyR mRNA and a distinct ~ 10 kb IP3R mRNA in all samples. IP3R mRNA levels were significantly increased in atria, but not in LV, of AF patients compared to controls.



In contrast, RyR mRNA levels in both atria and ventricles were not different between AF and control patients. We conclude that patients with AF demon-

MONDAY ORAL

strate selective up regulation of atrial IP3R Ca^{2+} release channel mRNA levels. Increased atrial IP3R expression may contribute to the molecular substrate for AF arrhythmogenesis.

11:45

703-6 Vascular Endothelial Growth Factor is Upregulated by Hydrogen Peroxide in Vascular Smooth Muscle Cells and Overexpressed in Atherosclerotic Tissue

J. Ruef, Z.Y. Hu, L.-Y. Yin, F. Li, G.N. Rao, M.S. Runge, C. Patterson. *University of Texas Medical Branch at Galveston, Galveston, TX, USA*

Neovascularization and vascular cell mitosis are hallmarks of neointimal formation in atherosclerotic plaques and restenotic lesions. Vascular endothelial growth factor (VEGF) promotes neovascular growth, whereas oxidative stress is a potent factor in vascular proliferation. To investigate the mechanisms of neovascular formation we treated rat vascular smooth muscle cells (VSMC) with hydrogen peroxide (HP). Northern blot analysis demonstrated a dose-dependent (20 to 500 μM) and time-dependent (0.5 to 6 h) increase in VEGF mRNA with a maximum of 10-fold at 3 h (200 μM HP). As determined by immunoblotting and ELISA, VEGF protein expression and secretion were similarly increased. Human umbilical vein endothelial cells (HUVEC) were treated with conditioned medium from VSMC incubated with 200 μM HP for 4 h. DNA synthesis, measured by thymidine incorporation, was increased by 1.7-fold compared to controls, an effect that was blocked by neutralizing anti-VEGF antibody. The effect of HP on VEGF expression and induction of HUVEC DNA synthesis by conditioned medium could be inhibited by the tyrosine-kinase inhibitor, genestein. HP had no effect on the expression of the VEGF receptor KDR/flk-1 in HUVEC. Immunohistochemical staining of aortic sections from balloon-injured baboons demonstrated increased VEGF expression in the neointima compared to controls. Together, our data suggest that HP is an indirect inducer of endothelial cell growth through release of the angiogenic factor VEGF from VSMC and that oxidative stress is a factor in atherosclerotic neovascularization.

704 Dobutamine Stress Echocardiography

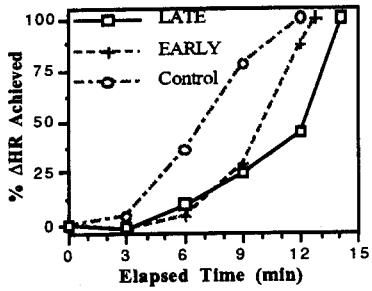
Monday, March 17, 1997, 10:30 a.m.–Noon
Anaheim Marriott, South Hall

10:30

704-1 Reduced Test Time by Early Identification of Patients Requiring Atropine During Dobutamine Stress Echocardiography

T.J. Lewandowski, W.F. Armstrong, D.S. Bach. *University of Michigan, Ann Arbor, MI, USA*

Atropine (AT) is used in patients with inadequate heart rate (HR) response during dobutamine stress echocardiography. The purpose of our study was: 1) to prospectively test an algorithm for early identification of pts requiring AT, and 2) to study the effect of early AT administration on test duration. Two-hundred and nineteen pts were randomized to receive either conventional AT at peak infusion if HR < 100 (LATE) or AT beginning at the end of the second stage (20 μg/kg/min) if HR < 70 (EARLY). AT was required in 86 pts; 35 received AT LATE and 51 were randomized to receive AT EARLY. Of the 51 EARLY pts receiving AT, 82% met criteria for and received AT early, while 18% were missed by the algorithm and received AT at peak. AT dose was 0.80 ± 0.38 mg in EARLY pts and 0.39 ± 0.20 mg in LATE pts. Despite attaining a greater ΔHR, test duration was reduced 1.3 min (9%, p = 0.01). The % of ΔHR achieved vs elapsed time was plotted for both groups and compared to 133 pts who did not receive AT.



In conclusion, pts who require AT during dobutamine echocardiography can be reliably identified before peak stress. Early AT use provided a more balanced physiologic stress and reduced total exposure to dobutamine, thus decreasing test duration and potentially increasing test efficiency.

10:45

704-2 Atropine is not Necessary for Dobutamine Stress Echocardiography

P. Desai, K. Conners, R. Conant, S. Shapiro, L. Ginzton. *Harbor-UCLA Medical Center, Torrance, CA, USA*

Dobutamine (DOB) is used to detect myocardial ischemia. When DOB fails to reach the target heart rate (HR) atropine is often added. *Hypothesis:* atropine will not increase the frequency of ischemia during DOB stress echocardiography (DSE). *Methods:* From January, 1994 to August, 1996, 239 patients (pts) had DSE to diagnose ischemia. DOB was given (5–50) mcg/kg/min. Atropine (0.5–2.0 mg) was added at peak DOB if the HR was <85% of the age-predicted maximal HR. Images were recorded at baseline, at peak DOB and at atropine's peak HR. Wall motion abnormalities (WMA) were scored by 2 echocardiographers blinded to all other data. Ischemia was defined as new or worsening WMA. *Results:* 114/239 pts received atropine. Of these 70 tests were normal, and 44 abnormal. No tests negative with DOB became positive after atropine. Only 4 pts had worse WMA with atropine, changing the result from single-vessel (SVD) to multivessel (MVD) disease.

	Baseline	Peak DOB	Peak Atropine
HR	73 ± 15	113 ± 23*	130 ± 15**
Normal	74	70	70
Ischemic SVD	14	14	10 †
Ischemic MVD	22	30	34 †

* p < 0.001 vs Baseline ** p < 0.001 vs peak DOB † p = NS vs DOB

Conclusions: During DSE adding atropine does not add to the frequency or extent of WMA. Therefore, atropine may not be needed for DSE.

11:00

704-3 Frequency and Etiology of False Negative Results in a Large Unselected Patient Population Undergoing Dobutamine Stress Echocardiography

R. Yeleti, M. Al-Dalli, P. Brenneman, D.S. Segar, H. Feigenbaum, S.G. Sawada. *Krannert Institute of Cardiology, Indiana University, Indianapolis, Ind, USA*

DSE has demonstrated a high sensitivity for the detection of coronary artery disease (CAD) in selected patient populations. To determine the sensitivity and causes of false negative studies in a large unselected population, the results of DSE in 2898 patients were reviewed. *Methods:* Of the 2898 patients who underwent DSE, 565 underwent coronary angiography (CA) within six months of DSE. DSE was considered positive in the presence of a resting wall motion abnormality (WMA) or a stress induced WMA. Significant CAD was defined as a luminal narrowing of >50% in a major epicardial artery. *Results:* The sensitivity of DSE for detecting CAD in all 565 patients was 94% and the specificity was 82%. In the 222 patients without resting WMA, the sensitivity was 83% with 26 false negative exams. The CA of these patients were reviewed to identify potential causes for the false negative tests: 13 patients had lesions with >50% but <70% stenosis; 7 had lesions supplied by collaterals; 4 had disease in distal vessels; and 2 had isolated circumflex artery disease. Nineteen of the 26 had submaximal stress tests. *Conclusions:* In a large unselected patient population, DSE had reasonable sensitivity for the detection of CAD. Patients with false negative exams frequently had submaximal studies with mild to moderate CAD (<70% stenosis, distal disease, or lesions supplied by collaterals).

11:15

704-4 Submaximal Responses to Dobutamine Stress Are Unhelpful in Predicting Subsequent Cardiac Events

R. Ballal, M.-A. Secknus, R. Mehta, M.S. Lauer, T. Marwick. *Cleveland Clinic Foundation, Cleveland, OH, USA*

Exercise tests are considered submaximal (SMX) if pts do not attain target heartrate (ie <85% max age-predicted). The significance of a submaximal (SMX) response during dobutamine echo (DbE) is unclear. Of 1772 pts undergoing standard DbE (Db to peak 40 μg/kg/min with atropine to 1 mg) from 1991–4, 255 pts (14%) had a SMX response and were followed for 2 years.

Results: Pts with SMX were of similar age (62 ± 13 vs 64 ± 12 y) and gender (55% vs 57% men) to the remainder. Resting heart-rate (73 ± 13 vs 75 ± 14) and SBP (144 ± 26 vs 148 ± 27) were comparable. Pts with SMX-DbE demonstrated *ischemia (ISC)* in 46 pts (18%); 20 had coronary